

REMARKS

I. STATUS OF THE CLAIMS

Claims 18, 49, 51 and 52 are canceled. Claims 1, 4, 5, 11, 15, 19, 20, 46, 50, and 64 are amended. Claims 1-17, 19-48, 50, and 53-72 are pending.

Support for amended claim language is found throughout the specification and claims as originally filed. Support for “to an alcohol concentration of about 35% to about 70%” is found, for example, at page 8, lines 19-21; at page 11, lines 20-21; and at Claims 48 and 52 as originally filed. Language added to Claims 4 and 5 is found at page 33 of the specification, lines 21-22. Language added to Claim 50 is from now-canceled Claims 51 and 52. Applicants submit that no new matter has been introduced by the amended claims.

II. OBJECTIONS TO THE SPECIFICATION

The Action states an objection to the specification for lacking support for a first solid support and a second solid support as recited by d) – g) of Claims 50-53 and 57. See Office Action at pages 3-4. Applicant respectfully traverses.

Response

Applicants note that elements d)-g) were present in claim 50 as originally filed and original claims constitute their own description. *See In re Koller*, 613 F.2d 819, 204 U.S.P.Q. 702 (C.C.P.A. 1980).

Furthermore, elements d) through g) are supported by the specification and are repeated here followed by the page and line number of the specification, and the exact language, that provides support for each element:

- d) *applying the lysate/alcohol mixture to a first solid support*; See page 32, lines 23-25: “The four aliquots had 100 µl of 40%, 50%, 60%, and 70% ethanol added to each, **then were passed through glass fiber filters** as in the RNAqueous procedure (Ambion).” Emphasis added.

- e) *collecting flow-through lysate/alcohol mixture*; See page 32, lines 25-27: “The 20%, 25%, 30%, and 35% ethanol **solutions that passed through these filters (the flow-through)** was then adjusted to 55% ethanol final concentration by the addition of 156, 133, 111, and 88.9 μ l of ethanol, respectively.” Emphasis added.
- f) *adding to the flow-through lysate/alcohol mixture an alcohol solution*; See page 32, lines 25-27 as cited above for e): “The 20%, 25%, 30%, and 35% ethanol solutions that passed through these filters (the flow-through) was then **adjusted to 55% ethanol final concentration by the addition of 156, 133, 111, and 88.9 μ l of ethanol**, respectively.” Emphasis added.
- g) *applying the lysate/alcohol mixture to a second solid support*; See page 32, lines 27 – 28: “All four samples were **passed over separate glass fiber filter columns**.” Emphasis added.

Therefore, elements d) through g) are provided at page 32 in the exact order as recited in the claim. Glass fiber filters are used in element d) and separate glass fiber filters are used in element g). That a glass fiber filter is considered a solid support is found at page 9, lines 21-23: “Supports include, but are not limited to, beads, columns and filters. In further embodiments, the mineral or polymer support is a glass fiber filter or column.” Further support for first and second solid supports is found at page 10, line 28 – page 11, line 1: “Additional method steps include **passing the small RNA molecules through a GFF while binding only the larger RNAs**. In some embodiments, **the passed small RNA molecules are captured on a second GFF** and then eluted.” Emphasis added.

In addition, please note that **FIG. 7** is entitled “Partitioning to Second Column.” That columns are also considered solid supports is supported by the statement at page 9, line 21 cited above.

Therefore, full support is provided by the specification for the language of elements d) – g) of Claim 50. Claims 51-53 and 57 are directly dependent upon Claim 50. Therefore, Applicants submit that said claims have full support in the specification and respectfully request that the objection to the specification be withdrawn.

III. CLAIM OBJECTIONS

The Action states an objection to Claims 11-13 as being of improper dependent form. See Office Action at page 4.

Response

Claim 11 has been amended to recite “wherein the lysing solution comprises a detergent and a buffer.” Support for a lysing solution comprising a detergent and a buffer is present beginning at line 21 of page 37:

An equal volume was added to each consisting of either Lysis Buffer with no guanidinium (*0.1 M β -mercaptoethanol; 0.5% N-lauroyl sarcosine; 25 mM Na-citrate, pH 7.2*) or Lysis Buffer with 2 M GuSCN (2 M GuSCN; 0.1 M beta-mercaptoethanol; 0.5% N-lauroyl sarcosine; 25 mM Na-citrate, pH 7.2), creating solutions with a final [GuSCN] of 2 M and 3 M, respectively. Emphasis added.

Applicant believes Claims 11-13 are of proper dependent form in light of amended Claim 11. Applicant therefore respectfully requests withdrawal of the objections to Claims 11-13.

IV. REJECTIONS OF CLAIMS 12, 14-17, 46 AND 64 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 12 and 14 satisfy 35 U.S.C. §112, second paragraph

The Action states a rejection of Claims 12 and 14 under 35 U.S.C. §112, second paragraph, for indefiniteness regarding whether the concentration of the detergent or the buffer is a starting concentration before mixing in the lysing solution or the final concentration in the lysing solution. See Office Action at page 4.

Response

Applicant submits that amended Claim 11, in reciting “wherein the lysing solution comprises a detergent and a buffer” clarifies that the concentration of the detergent or the buffer in the lysing solution is a final concentration in the lysing solution. Claims 12 and 14 are

directly dependent upon Claim 11. Therefore, Applicants submit that said claims are not indefinite and respectfully request that the objections regarding Claims 12 and 14 be withdrawn.

Claims 15-17 satisfy 35 U.S.C. §112, second paragraph

The Action states a rejection of Claims 15-17 for indefiniteness as to whether the organic solvent extraction is carried out after a) or b). See Office Action at pages 4 - 5. Applicant respectfully traverses.

Response

Claim 15 has been amended to recite “further comprising extracting small RNA molecules from the lysate with an extraction solution comprising an organic solvent after lysing and prior to adding an alcohol solution to the lysate.” Support for language of amended Claim 15 is found at Example 1 where phenol-chloroform was added to lysate, the suspension agitated and 1.22 volumes of ethanol was added to the aqueous phase to a concentration of 55% ethanol. Further support is found at Example 3 where phenol-chloroform was added to lysate and, after agitation, the aqueous phase was removed and ethanol was added thereto.

Claims 46 and 64 satisfy 35 U.S.C. §112, second paragraph

The Action states a rejection of Claims 46 and 64 for indefiniteness for omitting essential steps. Further, the Action states a rejection of Claim 64 for omitting a lysis step. See Office Action at page 5. Applicant respectfully traverses.

Response

Claim 46 has been amended to include the phrase “a sample containing miRNA molecules” and Claim 64 has been amended to include the phrase “a sample containing small RNA molecules.”

The specification at page 7, lines 1-5, states:

Samples from which small RNA molecules may be isolated include any sample containing such molecules. The sample may be or contain cells, tissue, organs, or other biological sample. *Alternatively, the sample may be a reaction mixture, such as one in which small RNA molecules were produced, generated, or created by enzymatic, synthetic, and/or recombinant means.* Emphasis added.

Clearly, the specification provides for isolating small RNA molecules from a sample without using a lysis procedure. Applicant submits that Claim 64 as amended fully satisfies the requirements of 35 U.S.C. §112, second paragraph.

For the reasons set forth above, Applicant respectfully requests withdrawal of the rejections under Section §112, second paragraph.

V. REJECTIONS OF CLAIMS 4, 5, 63-72 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 4 and 5 satisfy 35 U.S.C. §112, first paragraph

The Action states a rejection of Claims 4 and 5 under 35 U.S.C. §112, first paragraph, for lacking description of how to obtain the percentage yield of small RNAs without knowing how to determine the total amount of small RNAs present in any cell. See Office Action at pages 5-6.

Response

Claims 4 and 5 are amended to add language from the specification at lines 21-22 of page 22 as follows: “as compared to a standard RNA preparative procedure using organic extraction and ethanol precipitation using 4 volumes of ethanol.” In light of support in the specification for such language, Applicant respectfully requests withdrawal of the rejection of Claims 4 and 5 under 35 U.S.C. §112, first paragraph.

Claims 63 and 72 satisfy 35 U.S.C. §112, first paragraph

The Action states a rejection of Claims 63 and 72 for introducing new matter, specifically wherein the first wash comprises ethanol at 70%, and the second wash solution comprises ethanol at 80%. See Office Action at page 6. Applicant respectfully traverses.

Response

The specification states at least 5 different instances the use of a first wash comprising ethanol at 70%, and a second wash solution comprising ethanol or alcohol at 80%. Those at least

5 instances are at lines 26-27 of page 12, lines 25-26 of page 27, lines 8-9 of page 30, lines 1-2 of page 33; and at lines 25-26 of page 35.

In light of support in the specification for the first wash comprising ethanol at 70%, and the second wash solution comprising ethanol at 80%, Applicant respectfully requests withdrawal of the new matter rejection of Claims 63 and 72.

Claims 64-72 satisfy 35 U.S.C. §112, second paragraph

The Action states a rejection of Claims 64 - 72 for lacking enablement for isolating small RNA from any sample. See Office Action at pages 6-7. Applicant respectfully traverses.

Response

Claim 64 has been amended to include the phrase “a sample containing small RNA molecules.” One of ordinary skill in the art would know if a sample contains cells that need lysis. If one of ordinary skill does not know if a sample contains cells, he/she can readily ascertain without undue experimentation whether a sample contains cells, for example, using microscopy. Clearly, reaction mixtures, cells, tissue, organs, or other biological samples fall within the term “sample” as recited by the specification at page 7 at lines 1-5. To require a lysis procedure in the claims would force Applicant to accept claims narrower than his invention in order to secure allowance of his patent. See, e.g., *Application of Sus*, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301,304 (1962):

The public purpose on which the patent law rests requires the granting of claims commensurate in scope with the invention disclosed. This requires as much the granting of broad claims on broad inventions as it does granting of specific claims on more specific inventions. It is neither contemplated by the public purpose of the patent laws nor required by the statute that an inventor shall be forced to accept claims narrower than his invention in order to secure allowance of his patent. *Application of Sus*, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301,304 (1962).

Claims 65-72 depend directly or indirectly upon Claim 64. In light of the above remarks, Applicant respectfully requests withdrawal of this rejection of Claims 64-72 under Section 112, first paragraph.

V. REJECTION OF CLAIMS 1-48 AND 50-72 UNDER 35 U.S.C. §103

Claims 1-48 and 50-72 are rejected as being unpatentable over a manual for micro RNA isolation (Stratagene, 2000) in view of Bost *et al.* (U.S. Patent Publication No. 2003/0138828 ('828)), in further view of Ekenberg *et al.* (U.S. Patent No. 6,218,531 ('531)). See Office Action at pages 7-14. Applicant respectfully traverses.

Response

Claims 18, 51 and 52 are canceled.

In *KSR International Co. v. Teleflex Inc.* U.S. No. 04-1350, April 30, 2007, the Supreme Court reiterated that the framework for determining obviousness under §103 it had set out in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 59 (1966) continues to "define the inquiry that controls" determination of obviousness or nonobviousness of the claimed subject matter. As set forth in *Graham*, obviousness under 35 U.S.C. §103 is a question of law based on factual inquiries: (1) the scope and the content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art, and (4) objective evidence of secondary considerations. In *KSR*, the Supreme Court cited that secondary considerations might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented.

Scope and Content of the Prior Art

Stratagene's Micro RNA isolation manual: At page 1 of the manual, the fourth full paragraph, the Micro RNA Isolation Kit is described as "a perfect system for extracting RNA from *very small samples*." The next sentence states: "When biological *samples are present in small amounts*" Therefore, the "micro" portion of the title of this manual refers to small sized samples, not isolation of RNA of small size. That the focus of this manual is RNA

isolation from small samples is further supported by the information at page 12 on the formaldehyde gel protocol which recites the size range of 400-2000 bases for most mRNA. Clearly, the manual does not contemplate isolation of small RNA molecules less than 400 bases.

Bost *et al.*: Bost *et al.*, at Example 13, cite isolation of DNA and the ability of high concentrations of RNA to inhibit binding of genomic DNA to silicon dioxide particles. No alcohol was used during binding. At Figure 13, the recovery of nucleic acid was visualized using 1% agarose gel electrophoresis. The smallest-sized band of the “Input” lanes (that may correspond to small RNA) appears not recoverable by either of the pH 6 or the pH 10 extractions under any of the RNA/DNA ratios. Similarly, Example 14 cites binding of RNA and DNA to silicon dioxide in the absence of alcohol. Figure 14 shows recovery of nucleic acid on a 0.8% agarose gel. No small RNA molecules appear to be present. Therefore, small RNA molecules are not isolated by the Bost *et al.* procedures.

Ekenberg *et al.*: At Examples 1 and 2 (columns 17 and 18), Ekenberg *et al.* teach homogenizing tissue using lysis buffer (step 1 of column 18), adding dilution buffer containing SSC and SDS (step 2 of column 18), centrifuging and transferring 500 µl of the supernatant to a fresh tube (step 3 of column 18), adding 235 µl of 95% ethanol to each tube (step 4 of column 18), transferring the mixture to a spin basket and isolating the RNA from each sample by either vacuum filtration or centrifugation (step 5 of column 18). That the Ekenberg *et al.* patent is focused on yield and purity (rather than size of RNA isolated) is evidenced by Tables 1-5 that provide such data.

Stratagene’s Micro RNA isolation manual in view of Bost *et al.* further in view of Ekenberg *et al.*: Bost *et al.* in combination with Ekenberg *et al.* do not remove the deficiencies of the Stratagene manual since none of the references, alone or in combination, cite procedures that achieve isolation of small RNA molecules.

Difference between the prior art and the claims at issue

Some of the differences between the prior art and the claims at issue include a method for isolating small RNA molecules from cells comprising: a) lysing the cells with a lysing solution to produce a lysate; b) adding an alcohol solution to the lysate to an alcohol concentration of

about 35% to about 70%; c) applying the lysate to a solid support; d) eluting small RNA molecules from the solid support; and, e) using or characterizing the small RNA molecules.

The level of ordinary skill in the art

According to KSR at page 17, a person of ordinary skill is a person of ordinary creativity.

Other criteria for establishing a *prima facie* case of obviousness relied upon include the so-called teaching-suggestion-motivation test which the Supreme Court found in *KSR* to be not necessarily inconsistent with the *Graham* analysis. For this test, three basic criteria are analyzed: the prior art reference (or references when combined) are analyzed for (1) a teaching or suggestion of all the claim limitations; (2) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; and (3) a reasonable expectation of success. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). See MPEP § 2142.

That the combination of Palmieri *et al.* in view of Ekenberg *et al.* '531 lacks a teaching or suggestion of the presently claimed invention is clear from the *Graham* analysis above.

Regarding motivation, the Supreme Court in *KSR* stated at Section C. p 15-16:

“In determining whether the subject matter of a patent claim is obvious, neither the particular motivation nor the avowed purpose of the patentee controls. What matters is the objective reach of the claim.”

Applicants submit that the combination of Stratagene's Micro RNA isolation manual in view of Bost *et al.* further in view of Ekenberg *et al.* does not render the subject matter set forth by the pending claims obvious, in part, because the combination of prior art references fails the “objective reach of the claims” test regarding “a) lysing the cells with a lysing solution to produce a lysate; b) adding an alcohol solution to the lysate to an alcohol concentration of about 35% to about 70%; c) applying the lysate to a solid support; d) eluting small RNA molecules from the solid support; and, e) using or characterizing the small RNA molecules.”

Further, the combination of art cited lacks any recognition of a problem to be solved regarding then-current methods of isolating small RNA. Therefore, the combination of art cited cannot and does not provide any motivation to develop methods for isolation of small RNA molecules.

The Supreme Court in *KSR* stated at Section C. p17:

“A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning. ... Rigid preventative rules that deny factfinders recourse to common sense, however, are neither necessary under our case law nor consistent with it.”

Applicants submit that comments of the Office Action clearly use hindsight to attribute properties to old RNA isolation methods that clearly are unfounded based on the combination of art cited.

For the above cited reasons, Applicants submit that the invention as set forth by the independent Claims 1, 42, 46, 48, 50 and 64 is patentable under U.S.C. §103 and respectfully requests that the rejection be withdrawn. An essential characteristic of a proper dependent claim is that it shall include every limitation of the claim from which it depends. Therefore, a dependent claim is allowable when the claim from which it depends is allowable. Claims 2-17, 19-41, 43-45, 47, 53-63 and 65-72 are directly or indirectly dependent upon Claims 1, 42, 46, 48, 50 or 64. Therefore, Applicants submit that said claims are patentable also and respectfully request that the rejection under U.S.C. §103 be withdrawn.

VI. CONCLUSION

Applicants believe that the present document is a full and complete response to the Action dated August 23, 2007. The present case is in condition for allowance, and such favorable action is respectfully requested.

The Examiner is invited to contact the undersigned Attorney at (512) 536-3167 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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